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Seed Corn Infection with *Fusarium moniliforme* and its Relation to the Root and Stalk rots

BY

W. D. VALLEAU

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INTRODUCTION

Altho the root and stalk rots of corn have been known to exist for many years, our knowledge concerning their nature the causal organisms and the method of infection is very meager.

The root and stalk rots are widespread in the corn-growing sections and when fields are carefully inspected, an extremely high percentage of the plants are found to be infected. The results of other investigators (8, 7) indicate that a portion of the infection may be the result of seed-borne organisms and that elimination of the diseased seed will control this portion of field infection. The source of the remainder of field infection has not hitherto been ascertained, but it must be either from the soil or from more extensive seed infection than we have supposed to exist.

The writer wishes to present evidence on the extent of infection of seed corn grown in various parts of Kentucky and several other states, and its possible bearing on the high percentage of root and stalk rot infection found in the field. This report includes a study of over three hundred ears of corn representing the varieties Boone County White, Reid's Yellow Dent, Hickory King, Iowa Silver Mine, Big Nubbin and several local varieties grown in Kentucky; Large Mexican June and Hastings' Rockdale from Georgia; Huffman, Paymaster, Hickory King and Webb's Improved Watson, from Tennessee; Mosby's Prolific from Mississippi; Pride of Saline, Kansas Sunflower, Hildreth Yellow Dent,

Midland Yellow Dent and Commercial White, from Kansas; St. Charles White and Commercial White, from Missouri; Rustler and Minnesota No. 13, from Minnesota, and an unknown variety from Arkansas. The sweet corn varieties, Stowell's Evergreen, Peep-o-day and De Lue's Golden Giant, grown in Kentucky, have also been tested for infection.*

HISTORICAL REVIEW.

The occurrence of certain organisms causing kernel and cob rots has been noted from time to time. Sheldon (13) described a Fusarium of corn in Nebraska which was thought to have some etiologic significance in relation to forage poisoning. This organism was found on rotting corn from several farms visited and was described by Sheldon as *Fusarium moniliforme* n. sp. Burrill and Barrett (1) dicust an ear rot of corn due to *Diplodia zeae* and described in some detail three Fusaria commonly causing ear rots in Illinois. These they called Fusarium I, II, and III, respectivly. Garman (4) observed in Kentucky that "a pink mold (a Fusarium) is very common in our fields and causes many grains apparently sound to assume a pink color." He also noticed that "what appears to be the same fungus is found on corn that is germinating badly in the field." He advised that "such corn ought not to be planted." He (3) assumed that the elimination of the dicolored kernels would eliminate the infected ones and advised that show corn bearing pink kernels should be eliminated from a contest. He believes that the pink mold "gets to the kernels only by way of burrows made by the corn-ear worm and would thus probably be controlled by preventing the injuries of the insect."

Probably the most comprehensive piece of work which has been published on corn root and stalk rots is that of Pammel, King and Seal (10). They describe in detail root and stalk diseases of corn and sorghum, both of which they have proved to be seed borne. The diseases were said to have been known in western Kansas for several years and to have caused great damage in

* The writer wishes to express his appreciation of the assistance given by Professor E. J. Kinney in turning over to him his selected seed corn for study as to infection and in obtaining samples of corn from various parts of the country and for other valuable assistance and suggestions.

some seasons. Both diseases were found to be caused by Fusaria which were identical on corn and sorghum, at least from one locality. The causal organisms were described in some detail but were not named or identified with any known Fusaria.

Selby (12), in speaking of the root rot of corn, states that "Primarily the disease may be regarded as caused by a soil-infecting fungus which attacks the rootlets, thence invading the larger roots and stem of the corn plant. Once within the stem, it may advance upward and even grow out into the ear, causing the pink mold of corn."

Hoffer and Holbert (8) have pointed out that certain Fusaria are carried in the seed of corn and that ears bearing infection may be determined in the germinator and eliminated, thus controlling, to a degree, the corn root rots. They state (9) that "The common wheat scab organism, *Gibberella saubinetii* (Mont) Sacc., is probably the most common pathogene responsible for much of the root and stalk rotting of corn plants in the central states." The evidence on which this statement was based is not given in this abstract. Hoffer (7) pointed out that methods similar to those employed in the selection of disease-free field corn could also be used for sweet corn which is similarly affected by the root rot. In larger plantings of sweet corn, where it is practically impossible to germinate seed from each ear, he suggested the elimination of any showing physical defects—which consisted primarily in brown discolorations of the seed.

Hewitt (6) reports that in Arkansas "ear molds caused by Fusarium are serious," constituting "the limiting factor in the production of some varieties." The affection is believed often to follow ear-worm injury but may be quite serious independently. Rosen (11) mentions the presence of a root rot of corn in Arkansas which he believes to be due to a bacterial organism. He isolated from a discolored node of a diseased plant an organism which was apparently capable of causing a root rot when a root of a corn plant grown in a test tube was inoculated with it.

SEED INFECTION STUDIES

Methods

In the germination studies carried on, surface-sterile seeds were germinated on agar petri plates, in sterile and unsterile sand, in test tubes of sterile water and agar, and in sterile rag dolls. Seeds not surface sterilized, also, were grown in sand and rag dolls.

The method generally used in surface-sterilizing seeds was to soak them for about one-half minute in 50 per cent alcohol, followed by from ten to twenty-five minutes in mercuric chlorid solution—1 to 500. Variations from this method of treatment will be indicated where used.

The rag doll germinator has been used to quite an extent in these studies, as it is a simple method and the results obtained may be considered fairly accurate. Seed discolorations have been used in determining infection, as it has been found that if sufficiently high temperatures (80 to 85° F) are used, infected seed will usually develop pink, scarlet, wine or black discolorations at various places on the seed coats, but generally at the tip. These reddish discolorations, if present, are indications of infection; but their absence does not necessarily mean that the seed is disease free. Infected seeds planted in sand develop the discolorations to a marked degree at a temperature of 70 to 85° F.

In selecting seed from an ear for germination, care has always been taken to obtain as composite a sample as the number of seeds used would allow.

Preliminary Infection Studies

During the fall of 1919 a study was made of seed infection in seed from a badly infected field of yellow corn in which, at harvest time, hardly a stalk remained standing. The seed used in planting this field had been obtained from a seedsman, but its origin could not be determined. A portion of the land on which this corn was grown had been planted to potatoes for the previous two years, the remainder had been planted to hemp the previous year and the three preceding years had grown tobacco.

In an adjoining field, planted to the same crops for the past four years, but planted with a variety of corn known to do well in the locality, the crop was apparently normal when the two fields were examined in the latter part of September. The roots of all plants examined in the diseased field were found to be rotting and the original seeds, when found among the roots, were scarlet or purple.

Nine ears of corn were taken from the badly diseased field for examination. Ten seeds from each ear were surface sterilized by soaking in water fourteen hours, mercuric chlorid solution (1 to 500 in 50 per cent alcohol) two minutes, rinsed in two changes of 95 per cent alcohol and placed on agar in petri plates. At the end of eleven days observations showd a pink discoloration developing in the seed coats of these seeds and an organism growing into the agar from the discolored areas. In each case the organism was identified as *Fusarium moniliforme,* Sheldon, by the presence of Fusarium spores and the long, moniliform chains of spores which are characteristic of the species. These results indicate that this organism may be carried within the seed and suggest that it may have been the cause of the serious root infection of the plants in the field.

An attempt was made to determine the extent of seed infection within an ear known to be diseased. Seventy seeds were selected from a single diseased ear of Boone County White, at varying distances from rotting seeds. Each lot of 10 seeds was handled separately. They were surface sterilized and placed in seven agar petri plates and kept at room temperature. At the end of six days plate 1 showed no infection. Plate 2 showed no organism growing into the agar, but the root tips of some of the seedlings were brown and stunted. When these were removed and transferred to another plate of agar, an organism grew out of some of them, which proved to be *F. moniliforme.* In plate 3, five seedlings had stunted, brown-tipped roots, as in plate 2. An outgrowth from one of the seeds in this plate was identified as *F. moniliforme.* In plate 4, all seeds germinated, but the primary root rotted in two cases after only a slight growth and two other seeds rotted during germination. In plate 5, all were apparently healthy. Plate 6 was apparently

normal except for one seed, in which case the root tips were brown. An outgrowth from this seed proved to be *F. moniliforme*. Plate 7 was made up of 10 seeds which showed signs of rotting and bore Fusarium spores on their surfaces. Outgrowths of hyphae soon appeared from 5 of these and isolations from 3 were determined to be *F. moniliforme*.

At the end of ten days these lots were discarded. All seed from which *F. moniliforme* was isolated had been in contact with rotting kernels on the ear, except in the case of the single infected seed in plate 6, for which plate seeds were selected at least one inch from rotting kernels. These results would seem to indicate that infection of seed on an ear is more or less localized, but subsequent observations prove that the time given was not sufficient for all seed infection to have become apparent.

Selection of Disease-Free Ears

During the fall of 1919, seed selected for use in 1920 on the Kentucky Experiment Station farm was brought into the laboratory for the purpose of eliminating in the germinator the infected ears (8). A survey of the 1919 crop on the farm showed that more than 95 per cent of all the plants was infected to a greater or less degree with root and stalk rots, indicating the necessity for some means of control.

The first lot tested consisted of 95 ears of Boone County White grown on the Experiment Station farm at Lexington. They were selections from a breeding plot and represented the best ears in the plot. Ten normal-appearing seeds from each ear were germinated in rag dolls, in an incubator at 27°C. At the end of five days they were examined and detailed notes taken on the germination and discolorations of each seed. Of the 950 kernels examined, 615 showed pink, scarlet or purple discolorations in the seed coats. Seeds from 3 ears of the 95 showed no discolorations. The roots on 479 seedlings were stunted, twisted and the tips brown. Rotting lesions were present on many of the roots. Great variability existed in the extent of discoloration on the various lots of seed. In some lots all seeds were badly discolored, with hyphae growing from the

tips of the seeds or from injuries in the seed coats; in others only an occasional seed was slightly discolored. Whether these variations in extent of discoloration of the seeds can be used as an indicator of relative resistance, remains to be determined.

A second lot of seed tested included the 95 ears reported on and 44 ears of Reid's Yellow Dent. Five seeds from each ear were planted in a sand germinator. Clean river sand, which had not been used for any purpose previous to this test, was used. The root development in the sand was much better than in the rag dolls. When the plants were about 6 inches high they were dug and the condition of the roots and seeds noted. Of a total of 475 seeds of Boone County White planted, 443 were recovered, mice having destroyed the remainder. Of those recovered, 314 bore pink or scarlet discolorations and 81 showed black stripes in the seed coats. The remaining 48 were partially brown or showed no discolorations. Lesions were present on the stems or roots of 172 seedlings, where these parts were in contact with their respective seed coats. It was impossible, toward the end, to separate all of the plants from a given ear from the remainder, because of the rapid root growth; therefore seed from only the first 60 ears were recorded separately. The remainder were dug and washed together and then separated and individual notes taken. Of the 60 ears separately recorded, some kernels from each but one showed pink or purple discolorations. The kernels from the one were marked with black stripes.

Of the 220 seeds of Reid's Yellow Dent planted from 44 ears, 197 seeds or seedlings were recovered. As the roots of these plants were badly tangled, they were dug in a mass and the sand washed from the roots, after which they were carefully separated and examined. One hundred and seventy-six seeds bore scarlet or scarlet and black discolorations, while 21 bore only black markings on the seed coats. Lesions were present on the stems or roots of 125 seedlings, where these parts were in contact with their infected seed coats. Rotting portions of roots were removed from 12 plants, washed in sterile water and placed in a petri dish. When examined several days later, all showed the typical moniliform chains of spores of

F. moniliforme. The same organism was identified on 4 seeds similarly handled. There seems little doubt that practically all of the 139 ears examined bore infected seed.

The germination in the 95 ears of Boone County White was 100 per cent in 77 ears, 90 per cent in 14 ears, 80 per cent in 3 ears, and 40 per cent in 1; while the seedlings in the sand germinator were vigorous, for the most part, in both the Reid's Yellow Dent and Boone County White. Apparently infection, unless very severe, does not affect germination and affects the vigor of the plant in its early growth only to a slight degree.

A third lot tested consisted of 55 ears of Reid's Yellow Dent, 44 of which had previously been tested in sand, in lot 2. Ten seeds from each ear were germinated in sterile rag doll germinators. Three hundred and sixty-eight developed reddish discolorations in the seed coats. These seeds were from 53 of the 55 ears tested. Seeds from only two ears showed no discolorations, but the seedlings from one of these had lesions on the roots. There was a great deal of irregularity of root development in the various lots, numerous roots being stunted and brown tipped. Many roots had rotting areas. Five stunted, brown-tipped primary roots which were bearing many laterals, were immersed in mercuric chlorid solution, 1 to 1000, for one minute, washed with distilled water and planted in agar plates. Four of 5 roots so treated were found to be infected with *F. moniliforme.*

A fourth lot of seed studied consisted of 19 ears of Reid's Yellow Dent, 17 ears of Peep-o-day sweet corn, 3 ears of another sweet corn, a lot of shelled seed of Stowell's Evergreen grown in a city home garden, 2 ears of 1918 Reid's Yellow Dent, 3 ears of Hickory King, 3 ears of Boone County White and 6 ears of yellow corn from a field badly infested with root rot. Five seeds from each ear were surface sterilized, washed in sterile water and placed in autoclaved rag doll germinators After germination, 169 of the 270 seeds were found to have developt a scarlet or purple discoloration. These seeds were from 49 of the 54 samples tested. *F. moniliforme* was found growing from the tips of some of the five lots of seed showing no discoloration.

It was identified a total of 34 times in association with the discolored areas on seeds from the 54 samples.

Thus far, of a total of 204 ears examined, 194 have proved to be infected.

In view of the fact that ear infection in this locality is very close to one hundred per cent, the selection of disease-free ears in the germinator seems to be out of the question as a means of control of corn root rot. This raises the question as to whether the percentage of ear infection may not generally be higher than has been suspected.

From the results which we have obtained, it appears that elimination of only those ears indicated by rotting plants or seeds, in the germinator run for a period of 7 or 8 days, will result only in the elimination of those ears in which the seeds are so badly infected that rotting begins practically as soon as growth commences. It will not, however, eliminate those in which infection is slight (or, more probably, those which are resistant to rapid spread of the organism) and in which no rot develops in the period of time given, but which will, if grown in sand for a longer period of time, develop discolorations in the seed coats and often a severe rotting of the roots and stems which are in contact with the seed coats.

The following results will illustrate this point as the seeds used were taken from an ear which would show practically no signs of infection other than seed coat discolorations, if grown in a germinator for a period of about a week. Fifty seeds were taken from an ear in which 100 per cent of the seeds had been found to be slightly infected with *F. moniliforme* in two previous trials. These were planted in sterile sand. When the plants were approximately 12 inches high, they were carefully removed from the pot, the roots washed clean and an examination made of each plant. Of the 50 planted all proved to be infected, as indicated by the presence of pink, red or purple discoloration in the seed coats. When cross sections of the plantlets were made at various places thru the cotyledonary node, the vascular tissue in only 2 was found to be discolored. The remainder were normal as far as the vascular area was concerned. The scutellum was discolored in 4. Thirty-seven seed-

lings had lesions on their roots and 22 on their stems where these parts were in contact with infected seed coats. Rot from a stem or root had spread slightly into the soft parenchyna tissue of the cotyledonary node in the case of 10 seedlings. Such seedlings, having only a slight infection within the seed coats, if dug when 3 inches high, will generally show either slight or no signs of infection. Discoloration of the seed coats may or may not have developt and the hyphae within the seed coats usually will not have grown out into the sand enough to cause an infection of the stem or roots. By far the greatest part of in fection of seed corn is of this type. Most plants from an ear of this type will receive a vigorous start in the field, but later the roots may become infected due to the outgrowth of hyphae from the seed coats, which will spread to other roots as they are produced. That the hyphae do grow out and cause infection in this manner was shown when this lot of seedlings was dug and the roots washed. It was found that masses of sand from 1-2 to 1 inch in diameter often clung to the seeds. The sand particles were cemented together by the hyphae growing from the seed. Such roots or stems as were present in these masses were generally rotting. It is also quite common to observe hyphae growing up from the seeds to the surface of the sand, producing a white weft on the surface, if the seedlings have been left undisturbed in the sand germinator 10 days or more. (Fig. 1.) These areas are often more than 2 inches in diameter.

Further evidence on the injury to seedlings from seed-borne infection is presented in Table I. The seed used were from Boone County White ears 1 to 69, reported on previously in the first and second large lots of seeds tested. Twenty seeds were selected at random from each ear and planted in clean, but not sterile sand. At the end of about eighteen days the plants from each ear were cut out of the sand, retaining only the roots for a distance of about 1 1-2 inches, the remainder being cut off. The plants and seeds were washed and examined for seed discoloration, injury to roots and stem and discoloration of the vascular tissue of the cotyledonary node. It was believed that if the original infection was within the germ this portion of the

plant would be the first to rot, while if it were only in the seed coats, as we assume, then rot of the base of the stem must proceed from without and the vascular tissue would be the last to become discolored.

Figure I. Ear to row tests in a sand box showing the result of seed-borne infection with F. moniliforme, at the end of 15 days, on seedlings from 2 ears of Boone County White. Ear 451 (left) gave a reading, when the plants were dug at the end of 21 days, quite similar to ear 60, Table I, with 8 plants entirely rotted. Ear 454 (right) gave a reading nearly identical with ear 24, Table I, but with one hundred per cent. infection in the seeds. The differences shown in the photograph and in the readings probably represent differences in resistance.

TABLE I.

The result of seed infection with *F. moniliforme,* Sheldon, on corn seedlings grown in a sand germinator eighteen days. Twenty seeds from each of 69 ears of Boone County White were used.*

Ear No.	Germination Per Cent	Seed discoloration No. of Seeds	STEM ROT		ROOT ROT				Rot of Vascular Tissue in Cotyledonary node	Total Plants With Rotted Parts
			⅛ Inch or More	Slight or Lesion on Side	Primary	Paired Seminals		Fourth Seminal		
						No. of Roots	No. of Plants Affected			
1	100	19	0	13	2	19	11	1	0	15
2	100	20	1	8	1	6	5	1	0	11
3	95	20	0	12	1	10	7	2	0	13
4	100	20	1	15	8	12	9	7	0	17
5	100	20	0	15	13	17	10	11	0	20
6	100	20	5	12	10	17	13	12	0	18
7	100	20	0	6	1	11	7	0	0	10
8	100	20	2	11	6	4	3	12	0	16
9	100	20	0	15	6	15	10	7	0	17
10	100	20	1	10	7	15	12	5	0	18
11	100	18	3	7	8	16	10	5	0	16
12	100	20	0	18	18	24	12	12	0	19
13	95	20	3	10	8	14	9	17	0	19
14	100	20	1	10	15	21	14	11	1	19
15	100	20	6	7	7	10	7	14	0	19
16	100	20	4	14	12	26	17	15	0	20
17	90	20	10	8	13	33	17	12	2	17
18	100	20	2	13	6	26	15	4	1	16
19	100	18	0	8	10	16	9	5	0	15
20	100	20	1	4	9	9	6	6	0	13
21	100	20	3	3	1	8	5	4	2	10
22	100	20	3	13	1	23	14	15	0	19
23	100	20	2	13	0	10	6	8	1	16
24	95	20	3	7	1	6	3	4	0	12
25	100	20	2	9	0	11	8	8	0	15
26	100	20	0	13	0	23	14	14	0	19
27	100	20	4	11	0	21	13	7	0	17
28	100	20	3	4	0	7	5	3	0	10
29	100	20	0	12	0	18	10	9	0	16
30	100	20	0	10	0	6	5	2	0	12
31	100	20	2	4	1	15	9	1	1	11
32	95	13	0	4	0	4	3	0	0	4
33	90	19	8	9	4	16	9	5	5	17
34	100	20	6	7	0	33	18	9	0	19
35	100	20	1	13	1	9	5	5	1	18
36	75	20	6	5	2	22	13	9	0	15

Ear No	Germination Per Cent	Seed Discoloration No. of Seeds	STEM ROT		ROOT ROT					
			⅛ Inch or More	Slight or Lesion on Side	Primary	Paired Seminals		Fourth Seminal	Rot of Vascular Tissue in cotyledonary node	Total Plants With Rotted Parts
						No. of Roots	No. of Plants Affected			
37	95	19	3	13	8	21	13	11	0	18
38	100	20	1	8	3	9	7	0	1	13
39	100	20	1	5	1	6	5	1	1	10
40	95	20	2	14	2	23	14	2	1	18
41	75	20	9	6	6	25	13	9	3	15
42	100	20	0	0	2	1	1	0	0	3
43	100	20	16	2	6	34	18	9	0	19
44	95	19	1	5	0	8	6	1	0	7
45	85	20	3	10	0	21	13	2	0	15
46	100	20	3	12	0	16	11	0	0	15
47	100	20	1	16	6	37	19	2	1	20
48	100	20	4	13	2	12	8	2	0	19
49	100	20	1	11	0	6	5	4	0	15
50	100	20	4	6	4	12	7	2	0	12
51	100	20	3	12	3	18	12	4	0	18
52	100	20	2	2	8	6	5	7	0	11
53	100	20	1	15	0	12	10	8	0	20
54	100	20	0	7	1	20	11	3	0	16
55	95	20	1	16	3	16	9	16	1	19
56	95	20	9	5	4	23	14	8	0	16
57	95	20	2	5	1	6	4	1	0	14
58	100	20	2	10	0	16	11	1	0	14
59	100	20	2	15	10	24	14	12	0	18
60	100	20	15	5	16	34	17	12	1	20
61	100	20	6	13	3	17	9	4	0	19
62	90	20	8	9	1	25	15	6	0	18
63	100	20	2	9	1	15	9	1	0	14
64	100	20	1	4	0	7	4	1	0	7
65	100	20	2	13	0	9	6	1	1	15
66	100	20	8	8	0	33	17	6	0	18
67	95	20	1	9	1	12	9	0	0	12
68	100	20	5	7	0	24	14	2	0	18
69	95	20	4	10	1	23	14	2	0	16
Total per cent	97.8	98.9	14.9	47.0	19.2	81.4	49.8	29.1	1.7	76.8

* The material presented in Table I. represents the results of the first 69 ears of a lot of 337 ears which were being studied for relative resistance. The remainder have since been completed. In the entire lot no disease-free ears were discoverd and practically 100 per cent. infection of kernels was found, as indicated in the table.

The results give further evidence on the extent of infection of ears and of seeds within the ear, as shown by discoloration. The injury to the young plantlets, however, gives more conclusive evidence than seed discoloration, of the actual extent of ear infection. It will be noticed that no ears have produced entirely disease-free seedlings, altho the extent of injury to the plants from a few ears was slight. It was evident in examining the plants that infection had not spread from one row of seedlings to another to any extent, because of the presence of the discolored areas in the seed coats and because of the fact that the lesions were, in most cases, only present where the plants were in direct contact with the infected seed coats Numerous paired seminal roots were observed which were curling about under the seed coats unable to break thru; with few exceptions these were rotting. In several instances permanent roots growing down thru the infected area were found to be badly rotted. The fact that very few seedlings of the large number examined showed a discoloration of the vascular tissue of the cotyledonary node, altho the stems on many were badly rotted from contact with seed coats, indicates that infection is not borne within the germ to any extent but is carried nearly entirely in the seed coats. Experiments on seed treatment in which the germination has been nearly doubled in badly infected seed by seed-coat treatment suggests seed-coat infection rather than germ infection.

TEST TUBE CULTURES

It is realized that conclusions as to the extent of infection, if drawn from the rag doll and sand germinator tests alone, are open to the serious objection that contamination may have taken place. The writer feels confident, however, that the results obtained by this method indicate accurately the extent of ear infection. Seed infection is undoubtedly considerably higher than is shown by the rag doll or sand germinator methods, unless these are carried on for a sufficient period of time.

To determine, by more carefully controlled methods, the extent of infection in ears examined by the above methods, a single kernel was seleected from each of 50 ears of Boone

County White. Only normal kernels, as far as observation could determine, were selected. These were surface sterilized, washed in sterile water, then placed in sterile tubes containing a small amount of sterile water. At the end of four days, 2 seeds showed a slight pink discoloration at the tip; the remainder showed none. At nine days, 26 seeds had developt a discoloration. At the end of forty-four days, 11 seeds showed no discoloration, 7 a brown discoloration, 9 were pink at the tip, 9 purple tipped, while 14 were purple over all or more than half the seed. At this time, 10 seeds showed no outgrowth of hyphae; on 12 hyphae were just visible; on 20 they were plainly visible, while they had entirely overgrown the remaining 8 seeds. There was also a marked difference in the extent of growth from the seeds. Several did not germinate; the roots rotted as they emerged in some; others showed varying degrees of root injury, while a single one showed no apparent injury. An organism was isolated from 48 of these tubes and identified as *F. moniliforme* in 41 cases. The other seven cultures have not sporulated but have the same characteristic interwoven strands of hyphae commonly found in *F. moniliforme*. As the writer has isolated two other distinct strains of *F. moniliforme* from corn, he believes these 7 cultures represent a third strain of the same species. These results, where a random seed from 41 of 50 ears showed definitely an infection with *F. moniliforme*, confirm the results obtained in the rag doll and sand germinators.*

In another trial 25 seeds from each of fourteen ears were thoroly coated with bordeaux mixture, allowed to dry and were then planted in steam-sterilized sand and left for a period of about 10 days. In this trial a very high percentage of the seeds from each of the ears developed deep purple discolorations in a

* Since sending this manuscript to press the writer has noticed a contribution by F. D. Richey, Jour. Am. Soc. of Agronomy, v. 12, No. 1, 1920, on "Formaldehyde treatment of seed corn." The results obtained by him confirm those of the writer as to extent of infection, as when a single kernel was used from each of 25 ears infection was found to be as high as 97 per cent. at the end of only six days, when grown in water cultures. When seeds from the same ears were treated for two hours in a solution containing 25 cc. of commercial formalin in 1 liter of water, and were then placed in a closed chamber for a period of 24.5 hours they still showed as high as 70 per cent. infection at the end of six days. The organism concerned was found to be a Fusarium and was thought to be carried as spores on the surface of the seeds.

small band near the tip of the seed. Sections showed these area to be filled with short septate, profusely branching hyphae. The untreated checks likewise develop the discolorations in the seed coats, but in these cases it was generally lighter in color and more diffused over the seed. These results merely add evidence as to the high percentage of seed infection and give further proof that infection is carried within rather than on the surface of the seeds. They further indicate what has since proved to be true in the case of a large number of ears, i. e., that the presence of a few infected seeds in an ear may be regarded as evidence that practically all seeds of the ear are infected and that infection is not localized within an ear. In these trials, ears have usually been taken which show no physical evidence of infection; nevertheless they include ears which show a high degree of infection in the rag dolls as well as some in which infection, when judged by color, is detected with difficulty in the rag doll. The results obtained on seed treatment will be reserved for a future publication.

ETIOLOGY OF PINK KERNELS

About 20 per cent of the ears of Boone County White which have been examined have some kernels with a pink crown or pink stripes. These are sometimes present in sufficient numbers to give a distinct pink cast to the ear. A microscopic examination of the discolored areas has demonstrated the presence of hyphae, and occasionally spores in them, between the seed coats. In order to determine the organism associated with the discolorations the following tests were performed. Ten pink-crowned kernels were selected from each of 10 ears. These were surface sterilized, washed in sterile water and placed in autoclaved rag doll germinators. Ninety-eight per cent germinated. Eighty-three of the 100 seeds developt deeper pink or purple areas and the roots of 22 seedlings were stunted and abnormal. Many rotting areas were present on the roots, but the number was not recorded. An examination of the discol ored areas, after germination, showed an abundance of hyphae between the seed coat layers. Sixteen root lesions were soaked

in a 1 to 1000 mercuric chlorid solution one minute, followed by 3 changes of distilled water, and were then planted in poured plates of potato agar. From one of these, *F. moniliforme* grew out. The others remained sterile (probably due to too long soaking in the mercuric chlorid solution). Seven discolored seeds were surface sterilized in the same manner as the root lesions and a portion of the purple area was removed from each and planted in agar. In each case *F. moniliforme* grew out. A plate was poured from a tube of agar into which had been dropped one of the purple-tipped seeds with an outgrowth of hyphae. Numerous colonies of *F. moniliforme* developt.

Further attempts to determine the organism associated with the pink areas confirmed the above results. In one case, 47 pink-crowned seeds were selected from as many ears, surface sterilized in 50 per cent alcohol followed by thirty-three minutes in 1 to 500 mercuric chlorid solution; after which the seeds were washed in sterile water, the crowns removed, using aseptic precautions, and planted in agar plates. From 6 of these *F. moniliforme* grew out. There were no other outgrowths from the seed coats. In a second lot, 45 pink-crowned seeds were selected from as many ears of Boone County White, wet 1 1-2 minutes in 50 per cent alcohol, 4 minutes in a 1 to 500 solution of mercuric chlorid, washed 3 times in sterile water and allowed to soak two and one-half hours in sterile water, and were then washed in another change of water before the crowns were removed and planted in plates of agar. Outgrowths were obtained from 18 seeds, 15 of which were definitely determined as *F. moniliforme*. Ten pink-crowned seeds were selected from an ear on which more than one-half of the kernels were discolored. These were immersed in 95 per cent alcohol forty-five seconds followed by fifteen minutes in 1 to 500 mercuric chlorid solution. They were then washed in several changes of sterile water, 5 were placed in tubes containing agar, and 5 in tubes containing a small amount of sterile water. Eleven days later, 3 of the seeds had germinated normally, the leaves reaching to the cotton plug in the test tube. One of these seedlings had many rotting areas on the roots, but only a slight brown discoloration near the tip of the seed. Another showed no seed dis-

coloration nor lesions on the roots. It seemed at that time disease free. Eleven days later, a purple spot developt at the tip of the seed. The third had no rotting areas on the roots but the tip of the seed was dark wine colored. Two seeds did not germinate but turned dark wine colored and were partially covered with an outgrowth of hyphae. The remaining 5 germinated, but the roots were short or entirely rotted. The tips of 4 of the seeds were wine colored. One seed showed no discoloration and no lesions on the roots except that the tip of the primary root was brown. After twenty-four days, cultures were made from these tubes. In every case a pure culture of *F. moniliforme* resulted. These details have been given to show the great variability in vigor of seedlings which may result from diseased seed and it also proves that it is difficult to tell from external appearances for many days after germination whether or not seeds are actually infected.

GEOGRAPHIC DISTRIBUTION OF F. MONILIFORME

Corn root and stalk rots are undoubtedly present in all of the corn-growing states and it is very probable that the causal organism is the same over the entire corn-growing section. With the assistance of Mr. Kinney, of the Department of Agronomy of the Kentucky Experiment Station, the writer has obtained seed corn from several counties in Kentucky and from the states of Tennessee, Mississippi, Kansas, Arkansas, Georgia, Ohio and Minnesota. The results of infection studies made on these lots of seed indicate that seed-corn infection with *F. moniliforme* is widespread. In all cases the tests were made with surface-sterilized seeds germinated in sterile rag dolls, except the two ears of Minnesota No. 13, seeds of which were grown in sterile sand, and the 6 ears of Rustler, seeds of which were grown both in sterile sand and sterile rag dolls. Of 42 ears from 12 counties in Kentucky, seeds from only 4 ears showed no pink discolorations when grown in the rag doll germinators. Two hundred and eighty-four seeds of the 373 tested developt the discolorations. *F. moniliforme* was identified in association with the discolorations on the seeds from 7 counties. No attempt was made to determine the organism on the other lots of seeds,

as the evidence is nearly conclusive that the reddish discolorations indicate infection with *F. moniliforme.*

Forty of 50 seeds tested, from a lot of shelled corn of the variety Mosby's Prolific from Mississippi, showed discolorations, with which *F. moniliforme* was found to be associated.

Eleven of 12 ears of the variety Huffman, 12 of 15 ears of Webb's Improved Watson, 20 per cent of a lot of shelled Hickory King, and 10 per cent of a lot of shelled Paymaster, all grown in Tennessee, were found to be infected, as indicated by the development of reddish, discolored areas on the seed coats. *F. moniliforme* was identified more than 15 times from these lots. Room temperature was used in all of these trials, which may account for the low percentage of infection which became apparent in some of the lots.

Five lots of seed from Kansas, including the varieties Kansas Sunflower, Pride of Saline, Hildreth Yellow Dent, Commercial White and Midland Yellow Dent, were all found to be badly infected. *F. moniliforme* was identified 27 times from these lots.

St. Charles White and Commercial White from Missouri were both infected to some extent with *F. moniliforme.* Large Mexican June and Hastings' Rockdale from Georgia were both found to be badly infected with *F. moniliforme.* Seeds from five ears of a yellow corn grown in Arkansas were found to be badly infected with *F. moniliforme.**

Seeds from three ears of Rustler and three ears of Minnesota No. 13 from St. Paul, Minnesota, have shown 100 per cent infection when grown in clean sand.

The widespread distribution of this organism on moldy corn in Nebraska was pointed out many years ago by Sheldon (13).

The organism causing the root and stalk rots in Iowa was not definitely identified by Pammel (10), yet the writer believes that it, too, is *F. moniliforme* because of its effect on the plants, the fact that it is seed-borne and could readily be iso-

* These seed were obtained from the farm of E. B. Whitfield, of Bauxite, Arkansas, on whose farm Rosen (11) made his observations in regard to a bacterial root rot of corn.

lated from corn seed, because of certain cultural characteristics (especially its appearance on alfalfa agar in a petri plate, fig. 10) and because of the wide distribution which the writer has shown *F. moniliforme* to have in the corn-growing districts.

RELATION OF F. MONILIFORME TO CORN ROOT AND STALK ROTS

In view of the wide distribution of *F. moniliforme* in seed corn in areas where root and stalk rots are prevalent, the question arises as to the relation of this organism to the root and stalk rots. That it is parasitic is indicated by its presence within the seed coats of most of our seed corn. Numerous isolations of the organism from rotting roots and stems of corn seedlings indicate that it is capable of attacking them and causing a rot under laboratory conditions. Spaulding (14) and Hartley, Merrill and Rhoads (5) have proved beyond reasonable doubt that it is a very virulent damping-off organism of coniferous seedlings. From studies which the writer has made on seed infection and from isolations from the rotting roots and stalks of corn plants in the field, he is inclined to believe that *F. moniliforme* will prove to be the primary cause of root and stalk rots of corn in Kentucky and in the other central and southern states. From field observations in localities where wheat and corn are grown near each other one might conclude that the wheat-scab organism was the primary cause of the stalk rots, because of the very common appearance, in the fall of the year, of the gibberella stage of this organism on stalks and exposed bracer roots of corn; but is the presence of *Gibberella sp.* an indication that it is the causal organism? The writer's observations, during the past fall, indicate that root and stalk rots may be present on practically all plants in a field while very little of the wheat-scab organism may be present. A field of corn on the Kentucky Experiment Station farm, which was isolated from wheat for more than a mile on the windward side, was very badly infected with root and stalk rots, while 2 examinations, one during the fall and another later in the winter, on the standing stalks failed to reveal the gibberella stage of the wheat-scab organism. Another field of corn on the same farm, within a quarter of a

mile, with wheat growing across the road from it, was likewise found to be infected with root and stalk rots, but in this case nearly 100 per cent of the corn plants bore the perfect stage of the wheat scab organism in the early fall. There appeared to be a direct relation between the presence of *Gibberella sp.* on the corn and the presence of scab-infected wheat in the near vicinity.

Isolations from new root lesions on corn plants in the field have yielded *Rhizoctonia sp.* in one instance, a Fusarium resembling *F. culmorum* in another, and in 13 cases *F. moniliforme.* The rootlets from which these iolations were made had been immersed, after washing in water, in 1 to 1000 mercuric chlorid solution for two minutes, after which they were washed in sterile water and planted in corn-agar plates. These results are not extensive enough to be conclusive evidence of the most general cause of corn root rot, but they show that *F. moniliforme* may be associated with fresh lesions on the roots of field corn.

F. moniliforme has been isolated from the vascular tissue of internodes of rotting corn stalks beyond the point of visible rot. In some instances, *Gibberella sp.* was fruiting on the nodes found to be infected, internally, with *F. moniliforme.* It would seem in these instances that *F. moniliforme* was the active parasite while *Gibberella sp.* was merely saprophytic on the outside of the stalks.

Rotting shanks, which on examination were found to be filled with hyphae yielded *F. moniliforme* in pure culture when portions were removed from the inside and planted in agar, using aseptic precautions.

Numerous stalks have been found on which are scaly masses of salmon-colored Fusarium spores. These were often closely associated with the fruiting bodies of *Gibberella sp.* Cultures from the fusarium spores and from an outgrowth of the immature perithecia of *Gibberella sp.* yielded, in the former case, *F. moniliforme* and, in the latter, an organism identical in cultural characteristics with the wheat scab organism. It is apparent that these two organisms may be closely associated on rotting stalks.

INOCULATION EXPERIMENTS

Inoculation experiments under controlled conditions should be resorted to in order finally to prove the pathogenicity of *F. moniliforme* to corn roots, when plants are grown under nearly normal conditions. Such inoculation experiments have been tried but were done before the writer was aware that it was nearly impossible to find disease-free seed. As a cosequence, the results are of little more value than those obtained by growing infected seed in sterile sand. They leave little doubt, however, that the organism is parasitic on the roots of corn. Several pots of sterile soil were prepared and four surface-sterile seeds planted in each. A spore suspension of *F. moniliforme* was poured over the seed of those to be inoculated; others were left as checks, while one pot was inoculated with a culture of *Giberella sp.* from corn stalks. The checks and those inoculated with *F. moniliforme* showed about equal development, while those inoculated with *Gibberella sp.* grew about 4 inches high, at which time the roots were found to be rotted to the surface of the ground. *Gibberella sp.* was reisolated from these lesions. From time to time the checks and those inoculated with *F. moniliforme* were dug. Both were found to have lesions on the roots in an area surrounding the seed. *F. moniliforme* was isolated from the lesions on checks and those inoculated, repeatedly. Whether all of the infections were from the seed or whether some were due to the inoculum applied, could not, of course, be determined. These results leave no doubt that *F. moniliforme* within the seed is capable of growing into the soil and causing infection on new roots. Inoculation of healthy leaves of growing corn seedling with *F. moniliforme* resulted in a rot of them on which the fruiting bodies of *F. moniliforme* were identified. No evidence has yet been obtained as to the ability of *F. moniliforme* to live over winter in the soil. Some preliminary results indicate that it does not survive in stalks left standing in the field over winter, but these results need confirmation.

If the organism described by Pammel (10) causing a root and stalk rot of sorghum, proves to be identical with *F. moniliforme*, it would be expected that a similar disease of sorghum would be present in Kentucky where *F. moniliforme* is so wide-

spread. Only a small amount of preliminary work has been done by the writer on sorghum-seed infection. The organism has been isolated twice from two lots of surface-sterilized seed planted in agar petri plates. *Macrosporium sp.* grew out from many of the seeds in one lot but appeared not to be pathogenic. As little injury has been observed to sorghum in the field, inoculation experiments to determine the pathogenicity of *F. moniliforme* toward sorghum seedlings, were attempted. Fifty seeds were surface sterilized in 95 per cent alcohol followed by mercuric chlorid solution, 1 to 1000, for three minutes. These were planted in sterile sand and a heavy suspension of spores of *F. moniliforme* poured over them. Some of the seedlings were dug from time to time until 5 inches high, but no injury to the roots could be noted. At this time another spore suspension of *F. moniliforme* was poured over the plants and the pot covered with a bell jar. In two days all of the plants but two had damped off and a heavy growth of *F. moniliforme* appeared on them for about an inch above the surface of the sand. These rotting plants were mixed into the sand and the pot resown and kept covered with a bell jar. At the end of eighteen days no damping off had occurred and the roots, when dug, were, for the most part, free from visible infection, altho a few showed slight lesions. The plants were kept at a temperature constantly above 70° F.

SUMMARY

1. Corn plants in fields examined about Lexington have been found to be infected with root and stalk rots to the extent of nearly 100 per cent.

2. More than three hundred ears of seed corn examined, from the states of Kentucky, Kansas, Arkansas, Missouri, Tennessee, Georgia, Mississippi and Minnesota, showed infection with *F. moniliforme.* No ears examined from these districts were found to be free from infection.

3. Pink crowns or stripes, on normally white kernels, are an indication of infection with *F. moniliforme.* The development of reddish or black discolorations in the seed coats of corn during or after germination, is an indication of infection with *F.*

moniliforme. The absence of discolorations is not conclusive evidence of freedom from infection.

4. Results on more than sixty ears show that the presence of any infected seeds on an ear may be considered as evidence that all the kernels are infected with *F. moniliforme*.

5. *F. moniliforme* is an active parasite and is capable of causing root and stalk rots of corn under laboratory and field conditions.

6. The seed germinator method of selecting disease-free seed corn has not proved practical with the seed studied, as a means of eliminating diseased ears. It was of value only in the elimination of some of those ears so badly infected that plants from them rotted badly in the early stages of growth.

7. Infection, unless very severe, has little effect on germination and on the vigor of the young seedling. The organism will eventually grow into the soil and attack the roots. The high percentage of seed infection may account for the high degree of infection of corn plants with root rot found in the field.

8. Inoculation experiments with corn seedlings are of little value until a method can be devised of ridding the seed of contaminating organisms or until a source is discovered from which disease-free seed may be obtained.

9. *Gibberella sp.* may or may not be present in a field badly infested with root and stalk rots. *F. moniliforme* appears to be the more active parasite when it and *Gibberella sp.* are associated on rotting stalks of corn.

10. Because of the high degree of infection of seed corn with *F. moniliforme* over much of the corn belt, it will probably prove to be the most common cause of root and stalk rots of corn.

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